

Amendments to the Specification:

Please replace the title appearing on page 1, line 1 with the following title:

Immunogenic ~~composition~~ Fusion Protein Comprising *Babesia* Polypeptides

Please replace the paragraph at page 8, lines 21-23 with the following paragraph:

NB: The NCBI protein- and nucleic acid sequence databases can be reached through the Internet at ~~http://www.ncbi.nlm.nih.gov~~ the U.S. Department of Health and Human Services' National Institutes of Health's National Library of Medicine's National Center for Biotechnology Information internet site, and their use for the purpose of obtaining a. o. nucleic acid- or protein sequences is well-known in the art.

Please replace the table (with heading and caption) at the top of page 9 with the following table (with heading and caption):

Table 1: Examples of hydrophobic peptides for use in the invention

| Donor protein | NCBI | | Peptide's location in donor | Peptide's aa sequence (from N- to C-terminus) |
|---------------|----------|-----------|-----------------------------|---|
| | acc. nr. | aa nr. | | |
| Melittin | AAK92098 | 1 – 21 | N-term. | MKFLVNVALVFMVVYISYIYA (SEQ ID NO. 13) |
| DAF | B26359 | 352 – 381 | C-term. | TSGTTRLLSGHTCFTLTGLLGT LVTMGLLT (SEQ ID NO. 14) |
| CWP 1 | BAA07193 | 219 – 239 | C-term. | GAKAAVGMGAGALAVAAAY LL (SEQ ID NO. 15) |
| MV HN | P35971 | 35 – 58 | Internal | PYVLLAVLFVMFLSLIGLLAIA GI (SEQ ID NO. 16) |
| HHV-4 EBNA-3C | S27922 | 281 – 300 | Internal | EENLLDFVRF MGVMSSCNSS (SEQ ID NO. 17) |

DAF = Decay accelerating factor (CD 55); CWP 1 = *Sacharomyces* cell wall protein 1; MV HN = measles virus hemagglutinin-neuraminidase; HHV-4 EBNA-3C = human herpesvirus 4, nuclear antigen EBNA-3C.

Please replace the paragraph at page 16, lines 6-11 with the following paragraph:

The vaccine, or the vaccine with additional immunoactive component(s) according to the invention may additionally comprise a so-called "vehicle". A vehicle is a compound to which the fusion protein adheres, without being covalently bound to it. Such vehicles are i.a. bio-microcapsules, micro-alginates, liposomes and macrosols, all known in the art. In addition, the vaccine may comprise one or more suitable surface-active compounds or emulsifiers, e.g. Span™ or Tween™.

Please replace the paragraph at page 21, lines 6-20 with the following paragraph:

The ligation mix was transformed into JM109 ~~supercompetent~~TM SupercompetentTM *E. coli* cells (Promega). These cells were plated on ampicillin containing agar plates, and colonies were checked for expression of Bd37 protein by protein mini-expression. Briefly, a small scale (5 ml) bacterial culture in LB medium was initiated by 10-fold dilution of an overnight culture, after 2 h incubation at 37°C with shaking, recombinant protein expression was induced by addition of 1 mM IPTG (Euromedex). After 3 h of induction, cells were harvested by centrifugation (15 min, 4000 xg) and lysed in 1 ml of denaturing lysis buffer (8 M urea, 1% v/v Triton X-100 polyethylene glycol octylphenyl ether, 50 mM Tris, pH=8). Lysates were sonicated for 2 minutes with 2 second pulse-pause cycles on ice, and centrifuged (15000 xg, 10 min). Clarified lysates were incubated 20 min on ice with occasional shaking in presence of 50 µl of NiNTA agarose resin (Qiagen). Loaded resin was washed thrice with 1 ml of washing buffer (8 M urea, 1% v/v TX-100, 50 mM Tris, pH= 6.3) and protein eluted with elution buffer (8 M urea, 1% v/v TX-100, 50 mM Tris, pH= 4.5). The presence of recombinant protein was assessed by SDS-PAGE in 12% poly-acrylamide gel, which was stained with Coomassie Brilliant blue (CBB) and by Western blot with anti-Histag monoclonal antibody (Qiagen).

Please replace the paragraph at page 24, lines 15-22 with the following paragraph:

E. coli M15[pRE4] cells containing the different pQE plasmid constructs were each cultured overnight in LB medium at 37 °C containing 100 µg ampicillin, 25 µg/ml kanamycin, and 0.01 % v/v antifoam 209 (Sigma). Next morning the culture was diluted 1:10 in fresh medium and cultured for an additional hour. Then expression of the inserted fragment was induced by addition of 1mM IPTG. Culturing was continued for 4 additional hours. Next cells were pelleted by centrifugation (4000 xg, for 20 min) and resuspended in Histag lysis buffer containing 1% v/v Triton X-100TM polyethylene glycol octylphenyl ether, 1 mg/ml lysosyme and 1 mM phenyl-methyl-sulphonyl fluoride (PMSF) (Sigma). Lysate was stored at -80°C until use.

Please replace the paragraph at page 24, lines 23-30 with the following paragraph:

After thawing, 500 U DNase I enzyme (Life Technologies) was added, incubated 20 min on ice, next the suspension was sonicated on ice for 2 minutes with 2 second pulse-pause cycles. The sonicate was centrifuged at 9000 xg for 20 minutes. The supernatant was filtered sequentially through 1.2, 0.45 and finally through 0.22 μ m filters (Pall Gelman, France). Finally the filtrate was separated on FPLC-Ni²⁺ HiTrap™ FPLC Ni²⁺-chelating columns (Pharmacia). The loaded column was washed with Histag lysis buffer supplemented with 20 mM Imidazole (Sigma). Finally the rec proteins were eluted in Histag lysis buffer containing 200 mM Imidazole.

Please replace the paragraph at page 24, line 34 to page 25, line 9 with the following paragraph:

E. coli BL21 cells containing the pGEX plasmid constructs were each cultured overnight in LB medium containing 100 μ g/ml ampicillin and 0.01 % v/v antifoam 209 (Sigma), at 37°C. The culture was diluted 1:10 in fresh medium and culturing was continued for an hour. Protein expression was induced by addition of 0.1 mM IPTG, culturing was continued for 3 additional hours. Cells were pelleted as described above, resuspended in phosphate buffered saline (PBS) containing 1 % v/v Triton X-100™ polyethylene glycol octylphenyl ether, 1 mg/ml lysosyme, and 1 mM PMSF. As described above, the lysate was stored at -80°C, thawed, mixed with DNase I, sonicated and centrifuged. The supernatant was purified over Glutathione-Sepharose beads (Sigma). The beads were washed with PBS/1% TX-100™ and rec protein was eluted in a buffer containing 50 mM Tris (pH 8) with 45 mM Glutathione (Sigma).

Please replace the paragraph at page 29, lines 11-15 with the following paragraph:

Two linkers: Bd37HG3'-forw, and Bd37HG3'-rev (see Table 2) were designed, that encode the C-terminal 20 amino acids of the Bd37 gene, which is a hydrophobic region with the

amino acid sequence: FAAVPSSLSAIVFGIIVSMF (SEQ ID NO. 18). The two linkers also comprised restriction sites: a 5' ClaI site and a 3' RsrII site, which form upon annealing of the two linkers.

Please replace the paragraph at page 29, lines 16-20 with the following paragraph:

The two linkers were annealed and ligated into ClaI-RsrII digested pFastBacHA5 plasmid, to construct plasmid pFastBacHA5-Bd37. This HA5-Bd37 construct now encoded AIV H5 protein with a C-terminal fusion of the hydrophobic C-terminus from Bd37 protein. The C-terminal amino acid sequence of this HA5-Bd37 construct is (starting at AIV-H5 amino acid 516): EISGVKLEFAAVPSSLSAIVFGIIVSMF (SEQ ID NO. 19).